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A NEW GENUS OF PARASITIC GASTROPODS.

HAROLD HEATH.

A number of gastropods are known, living parasitically upon the body of certain echinoderms, which retain the shell and typical internal organization so that their systematic position is readily established. On the other hand several endoparasitic species exist which have become so highly modified that they stand in much the same relation to the free-living forms as *Sacculina* to the typical cirripedes. Owing to the lack of any detailed information relating to their ontogenetic development the relationship of such forms is highly problematical. And furthermore it is difficult to accurately follow the stages of the phylogenetic metamorphosis which the body has undergone, and accordingly to establish the homologies of some of the principal organs. Schiemenz[1] in a very suggestive paper has attempted to construct a hypothetical animal connecting the least modified species like *Stylifer* on one hand with the highly degenerate types represented by *Entoconcha.* In several respects the animal here described resembles the hypothetical form and in a measure enables us to follow some of the changes which the more degenerate species have undergone.

My attention was attracted to this gastropod by my friend and colleague Dr. W. K. Fisher who discovered it in a species of starfish (*Brisinga evermanni* Fisher) taken by the U. S. F. C. Str. "Albatross" in the neighborhood of the Hawaiian Islands (sta. 3467) at a depth of 310 fathoms. It occupied the cœlomic cavity close to the base of one of the arms, producing a marked distention (Fig. 2, Pl. I.) of the body wall. The animal was un-

[1] P. Schiemenz, "Parasitische Schnecken-Kritische Referat," *Biol. Centralbl.*, Bd. 9.

attached and was put in communication with the exterior by a slit-like aperture 2 mm. long. The body is subglobular in form, distinctly bilateral, light yellow in color and measures 14 mm. by 11 mm. The external opening, communicating with the exterior through the aperture in the arm of the host, is in the mid line and is surrounded by prominent lips. These last named structures are covered with a firm cuticle produced into 16 pairs of interlocking teeth (Fig. 6, Pl. I.).

An examination of Fig. 1, Pl. I., will disclose the fact that the body proper, containing practically all the organs except the female reproductive gland, is overgrown by a great fold attached to the front end of the body, but elsewhere separated from it by a narrow slit-like space which communicates with the above mentioned fissure-like opening guarded by teeth. Anteriorly the ventral surface of the body is developed into a snout-like projection bearing the mouth opening and a small pair of tentacles (Fig. 1). More posteriorly the ventral surface, corresponding to the foot of free living forms, is somewhat flattened but lacks the usual high ciliated epithelium and gland cells. Still farther back the peculiar kidney forms the ventral surface behind which is the rectum borne on a papilla-like elevation. Some of these characters and others to be mentioned indicate distinctly gastropod relationships which have been retained in spite of parasitic habits.

The entire surface of the body is covered with a cuticular layer usually well developed on the ventral surface over the lobe-like projections shown in Fig. 8, Pl. I. In this last named region it becomes developed into numerous small papillæ each of which is penetrated by what is probably a nerve fiber (Fig. 7, Pl. I.). Within the animal the layer is much thinner and over the respiratory papillæ (p) and the adjacent regions is provided with numerous slender and apparently solid, hair-like processes.

The hypodermal layer consists of flattened cells, with large nuclei, separated at many points to allow muscle fibers to attach directly to the overlying cuticle. In addition there are many bipolar cells, one fiber passing distally into the above mentioned cuticular papillæ the other becoming lost in the subjacent tissue.

The mouth opening is situated upon the summit of a well-defined

proboscis which is concealed from view by a fold (not shown in figure) springing from its base. From the study of sections it is evident that this fold is of greater length than the proboscis and is not united along the median line posteriorly. Externally the fold is covered by a cuticular layer which is continuous over the proboscis and within the digestive tract itself as far as the radula. This last named organ is in a very rudimentary condition since it consists of but a single tooth (Fig. 5, Pl. I.) composed of some finely granular substance staining intensely in Delafield's hæmatoxylin. On each side of it are the openings of the salivary glands which are doubtless the homologue of the ventral pair in other molluscs.

The main portion of each salivary gland consists of a large sac located at the sides of the body proper as shown in Fig. 1, Pl. I. The component cells are highly vacuolated structures containing a faintly staining, granular secretion that is present also in the adjoining cavity. Ventrally, in close proximity to the outlet of this portion of the organ, the walls of the sac change abruptly in character and consist of more slender non-staining elements developed into low folds. Surrounding these cells are others of considerably greater height that form a papilla projecting into the cavity of the next division of the organ (Fig. 4, Pl. I.). This last named section is plain-walled and is composed of low columnar cells containing small quantities of a finely granular material, possibly a glandular substance differing from that of the larger sac. At the base of the proboscis the walls become produced into several small folds before entering the slender canal passing onward to its outlet at the side of the radula. Beyond the radula the pharyngeal or œsophageal epithelium becomes thicker and is attached to several muscle fibers acting as dilators and constrictors. The opening into the stomach is on the summit of a papilla whose general features are represented in text fig. A.

Opening into the œsophagus in close proximity to the stomach are two sets of glands, that may correspond to the dorsal salivary glands of other molluscs, whose position has shifted, though there is a possibility that they are strictly œsophageal products. Each group extends from the neighborhood of their outlet far

out into the pseudo-pallium, in some instances coming in contact with the hypodermal layer. So far as may be judged from the present specimen every cell possesses a slender ductule extending to its independent opening into the œsophagus. The secretion is colorless or of slightly pinkish tinge and in many cases is of less volume than the uniformly granular, strongly staining nuclei. In some instances the last named structures are more or less spherical, and again may be mammillated or formed of approximately eight globular masses as though formed by the incomplete fusion of as many chromosomes.

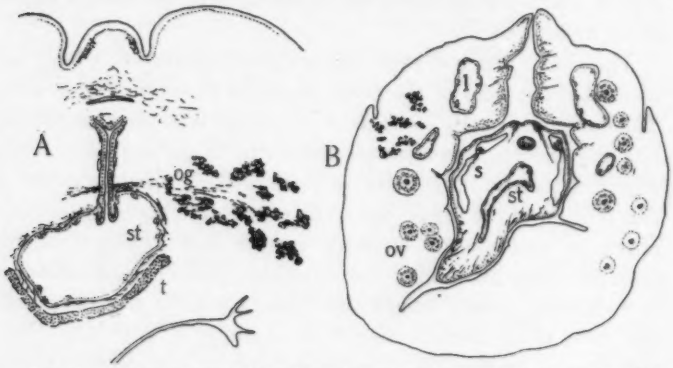


FIG. A. Section at level of œsophageal opening into stomach (*st*). *og*, œsophageal glands; *t*, testis.

FIG. B. Section corresponding to line *A*, Fig. 1, Pl. I. *l*, liver; *ov*, ova; *s*, salivary gland; *st*, stomach. The pleural pedal ganglion above (in figure) the stomach has, for the sake of clearness, been shifted slightly forward in Fig. 1, Pl. I.

The stomach is a voluminous sac extending throughout the greater part of the body proper. Its walls are composed of what appear to be two distinct types of cells (Fig. 3, Pl. I.) though they may possibly represent different stages of glandular activity. The more abundant form is almost cubical, highly vacuolated and contains a few slightly yellowish spherical granules. Among these are very much larger elements protruding some distance into the neighboring lumen and distended with a finely granular vacuolated material in which are a few spherical granules similar to those of the other type of cell. Opposite the level of the proboscis the stomach is expanded on each side to form a voluminous

pouch provided with a few secondary branches. These pouches probably are to be considered as representing the hepato-pancreas of other molluscs though the component cells differ in scarcely any essential respect from those of the main division of the stomach. At several points in the pseudo-pallium structures occur that strongly resemble the liver, but as they lack any definite connections it is impossible to claim that they were once united with the digestive tract.

The intestine presents the form of a cylindrical tube invested with numerous circular and longitudinal muscles attached by connective tissue and muscle bundles to the stomach and body wall. Its epithelial lining consists of relatively slender cells whose boundaries distally are difficult to determine owing to the large quantities of highly vacuolated protoplasm they contain. The outlet is guarded on each side by a large conical papilla covered with a thick cuticle fashioned near its tip into several slender filaments.

The stomach contains a small quantity only of a finely granular substance so that it is impossible to gain any insight into the methods of feeding and the nature of the food of this animal. It is probable that the proboscis and tentacles may be protruded through the external slit-like opening and organic substances may be picked up from the ooze as its host crawls about. It apparently absorbs little if any nourishment from its host though there may be some interchange of gases.

No sign of a heart exists in this species as is the case also with *Entoconcha*. A clotted substance abounds in the lacunæ among the various organs which doubtless represents blood. Groups of cells here and there may be corpuscles but their resemblance to connective tissue cells renders the determination uncertain.

As noted in a preceding paragraph respiration may be effected to a slight degree through the general body surface in contact with the cœlomic fluid of the host. The chief respiratory organs however appear to be the finger-shaped processes (Fig. 1, *p*, Pl. I.) attached to the posterior end of the body. With the exception of the hypodermal layer and a few muscle fibers passing in various directions from wall to wall or attached throughout their entire extent to the walls these organs are hollow and are probably

more or less distended with blood in a living condition. It is probable also that they may be extended into the neighborhood of the external opening or even protruded through it.

A problematic organ, which appears to be a kidney, is situated beneath the stomach anterior to the intestine (Fig. 1, *k*, Pl. I.). In this region the body wall is provided with a considerable number of outpouchings of varying size, though usually comparatively short, each of which is invested with a definite cuticular layer. In life all of these are probably filled with blood and are lined with a few connective tissue and muscle fibers some of which may span the cavity. With the exception of a few of the anterior projections the walls are provided internally with a very considerable number of relatively slender finger-shaped processes resembling those on the inner wall of certain prosobranch kidneys. They differ, however, in being invaginations of the body wall and are lined with a continuation of the cuticular layer covering the body generally. The cells of these minor processes are of moderate size, more or less cubical, though they often become flattened near the tip of the organ, and under high magnification have been seen in several instances to connect with a central lumen by means of delicate pores. Judging from these appearances the cells of each of these slender processes extract from the surrounding plasma waste materials, and pass them into the contained lumen from whence they make their way to the exterior.

While the ganglia are readily located the nerves are not sharply differentiated from the muscle fibers through which they pass and accordingly have been traced in a few cases only. The cerebral ganglia, united by a relatively long commissure, are situated in front of the pharynx and beneath the forward end of the stomach (Fig. 1, Pl. I.). Two connectives lead backward at the sides of the pharynx to the pleuro-pedal ganglionic masses placed about opposite the level of the posterior border of the proboscis. These last named ganglia are indistinguishably fused though they are distinctly paired and originate a very few small nerves that have been followed for a short distance only. One large branch on each side, arising from the anterior half of the nerve mass, passes dorsally and laterally and breaks up into three divisions. The first passes up into the large fold or pseudo-pallium close to the

large cavity, ventral to the body proper, that communicates with the exterior. The second pursues a course anteriorly in the pseudo-pallium and disappears from view among the large ova. The third, also in the pseudo-pallium, makes its way backward for a considerable distance but finally vanishes in several small muscle bundles.

Together with *Entoconcha* and *Enteroxenos* this species is monœcious and the ovary and testis are separate and distinct. The testis occupies a position lateral and ventral to the stomach about opposite the level of the pharynx, and is bilaterally symmetrical and gives evidence of being a paired organ. In the region concealed by the liver in Fig. 1. Pl. I., the organ is continuous across the mid line; but anterior to this point the unpaired division develops an extensive anteriorly directed pouch on each side of the stomach. The gland is in an immature condition, containing many spermatogonia but no later stages. Behind the unpaired section a duct of very large caliber arises, on each side of the mid line, with thin walls and a glandular, apparently ciliated, low ridge on its inner face. In this condition it continues to a point opposite the middle of the kidney where it contracts abruptly to a slender, thicker walled tube which is directed ventrally to open into the narrow slit-like space between the body and some of the kidney lobes.

The ovary is confined to the large fold enveloping the body, practically all of the space unoccupied by the liver being filled with large ova. These are suspended, almost precisely as in the chitons (see Haller's Fig. 328, Lang's "Lehrbuch," p. 366), in slender sacs, outpouchings of the germinal epithelium, and in a completed condition are surrounded by a follicle. No definite oviducts are present, the ova probably escaping by means of ruptures in the wall adjacent to the body proper.

The ova, fertilized either in or out of the body, may readily escape from the animal and from the host and doubtless in the free-swimming stage make their way to another host into which they burrow and take up their final position. The changes that ensue are wholly unknown but it is evident that the fold or pseudo-pallium, in this species at least, is not a portion of the foot. As may be seen in Fig. 1 it arises as a duplicature of the

body wall in front of the proboscis and may in reality represent a modified mantle, having been developed from such a type as now exists in the aspidobranchs.

Concerning the systematic relationships of this animal it is difficult to speak with certainty. From the paired character of the testis it appears to have been derived from some aspidobranch ancestor in which there were traces of the original bilateral symmetry. In its present condition the animal is perfectly symmetrical, but it does not follow that its immediate ancestors were such. Just as the modern more or less symmetrical lithodes have been derived from the asymmetrical hermit crabs so this species may have secondarily assumed its symmetrical condition.

The genus may be defined as follows:

Ctenosculum new genus. Body globular, bilaterally symmetrical, almost completely enveloped in a fold of the body wall. Proboscis and tentacles present. Two sets of salivary glands, radula reduced to one triangular tooth. Moncecious, testis and ovary distinct and separate, the latter occupying the fold. Parasitic in starfish, *Brisinga evermanni*, Hawaiian Ids. Type of genus *C. hawaiiense.*

C. hawaiiense new species. With characters of the genus.

Explanation of Plate I.

Fig. 1. *Ctenosculum hawaiiense* with left half of the pseudo-pallium removed. *c*, cerebral ganglia; *k*, kidney; *l*, liver; *o*, œsophagus; *p*, papillæ arising from body wall; *sg*, salivary gland; *st*, stomach.

Fig. 2. Cyst in starfish arm produced by parasite.

Fig. 3. Two types of cells of gastric epithelium.

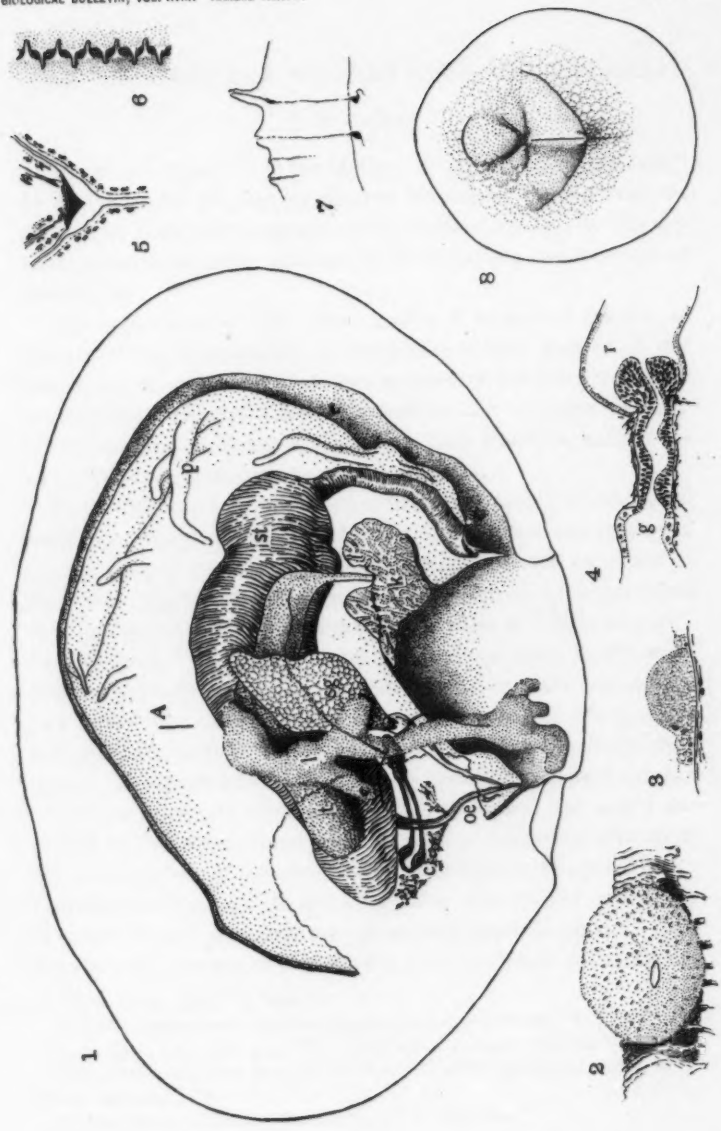
Fig. 4. Outlet of salivary gland (*g*) into reservoir (*r*).

Fig. 5. The single tooth of the radula with salivary ducts opening on each side.

Fig. 6. Cuticular teeth guarding the opening into space between body and pseudo-pallium.

Fig. 7. Papillate cuticle, from ventral surface of body, traversed by nerve fibers.

Fig. 8. *C. hawaiiense*, entire animal, ventral view.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THE OCCURRENCE OF AMITOSIS IN MONIEZIA.

C. M. CHILD.

In a recent paper, "On the Method of Cell Division in *Tænia*," by A. Richards,[1] the author records his failure to find amitotic division in *Tænia* and suggests on the basis of his results that my conclusions concerning amitosis in *Moniezia*[2] are due to errors of observation.

The appearance of this paper makes it necessary for me, in defence of my observations, to emphasize certain features of my own work, to which Richards has apparently not given adequate consideration, to add a few observations not included or only briefly referred to in my earlier papers and finally to subject his attempts at interpretation to critical analysis.

In the first place, it might seem that the reading of my statement concerning methods of fixation and precautions employed (I., pp. 89–93) would suggest to anyone that I had exercised at least some degree of care in order to avoid errors of observation of so gross a nature as the mistaking of a mass of "Nebendotter" for a nucleus. As I stated, fresh material was fixed by the most various methods during each of four successive years, and a large part of my time during this period was devoted to the study of the preparations, from which hundreds of camera drawings were made. The work was begun simply because the apparent absence of mitosis in certain regions of growth interested me, and I expected to find either some unusual type of mitosis or else short recurrent periods of mitosis. What I did find, however, led me to exercise the utmost care and to go over the ground repeatedly even after I had published my preliminary paper in 1904.[3] During the whole period of my work I was constantly searching for

[1] BIOL. BULL., XVII., 4, 1909.

[2] Child, "Studies on the Relation between Amitosis and Mitosis," I., BIOL. BULL., XII., 2, 1907; II., *ibid.*, 3–4; III., *ibid.*, XIII., 3, 1907; IV. and V., *ibid.*, 4. In later references to these papers in the text they will be designated simply by the Roman numerals I.–V.

[3] "Amitosis in *Moniezia*," *Anat. Anz.*, XXV., 22, 1904.

modified forms of mitosis as a possible basis for interpretation, but the frequency and almost diagrammatic clearness of the cases which I could interpret only as direct division, satisfied me that no such interpretation was possible. I have pointed out (I., pp. 91–2) the difficulties attendant upon the observation of amitosis in fixed material and the methods employed for giving the greatest possible certainty. Cases of apparent division which appeared in the slightest degree doubtful were not accepted as evidence. I have demonstrated many cases of apparent amitosis to others, both students and colleagues, who were able to see them with perfect clearness and had no doubt of their being what they seemed to be.

On pp. 312–313 of his paper Richards says: "I must protest against the balancing of results obtained from such unfavorable material as that which cestodes offer against such favorable objects for cytological study as for example the Orthoptera." This protest involves somewhat peculiar ideas of cytological study. Apparently, according to Richards, we must draw our conclusions from "favorable" material and leave the unfavorable aside. And apparently also because certain phenomena are clearly visible in favorable material, *e. g.*, the Orthoptera, we must conclude that the phenomena in other less favorable material are essentially similar. No field of biological science, and perhaps no field of science in general, has suffered to such an extent as has morphology from premature generalizations based on observation of one or a few forms. Indeed it is perhaps not too much to say that limited observation and premature attempts at generalization often go hand in hand.

As regards the "unfavorable" character of the *Moniezia* tissues I must disagree to some extent with Richards. With proper fixation, with sections sufficiently thin — most of mine were 2–3 micra — and with careful staining and extraction, the material is no more difficult to study, at least as regards nuclear phenomena, than many other tissues. I have seen hundreds of cases of apparent amitosis which were almost diagrammatic in their clearness when observed with a little care, though of course not as conspicuous as certain phenomena of mitosis in certain species and cells. If observation of fixed and stained material is

of any value at all as a means of discovering the visible phenomena of cell division, I can only conclude that direct division occurs in *Moniezia* as I have stated and that it can be seen readily enough by anyone who is willing to take sufficient care in preparation and observation.

Moreover, I have not, so far as I am aware, attempted at any time to "balance" my observations against those of others. The real point at issue is not my observations against those of others, but my observations against a hypothesis, which cannot be proved by observation, viz., the chromosome hypothesis, for the only real objection to the acceptance of my observations at their face value is the existence of this hypothesis. I do not wish to enter into fruitless discussion of the hypothesis, but merely to emphasize the fact that I have attempted in every way possible to make the record of my observations a record of actual fact. Such records cannot be controverted either by observations on another genus and species or by invoking a hypothesis which is far from being proved. It is interesting to note that Richards' paper is to a considerable extent devoted to "balancing" his observations against my own, although both according to him are made on the "unfavorable" cestode material.

Turning now to certain matters of detail, we may consider first certain points with respect to the parenchyma. My observations on amitosis began with a study of the neck-region, *i. e.*, the region between the scolex and the first visible proglottids. In this region the tissue of the body consists mainly of parenchymal cells, muscle fibers, the nerve cords and the nephridial canals. At the posterior end of this region the proglottids become visibly marked off one after the other, each including a certain portion of the tissue. In other words, the cells which constitute each proglottid either preëxist, or arise by the division of preëxisting cells in the neck-region, or else they must arise *de novo*. Examination of transverse sections at short intervals from the scolex backward into the proglottid region shows a very considerable increase in the number of nuclei, as I have determined by actual counts of the nuclei in the sections. In short there can be no doubt that new parenchymal nuclei are formed in this region, and when we consider the enormous number of proglottids formed

from a single neck-region and observe that the number of nuclei in the neck-region at any given time is sufficient for only a few proglottids it becomes evident that during the life of the animal an enormous number of new nuclei is formed in some way in this region. These facts dispose of Richards' conclusion that the parenchyma grows chiefly by the formation of "intercellular material."

Moreover, in view of these facts it is not in the least surprising that Richards has failed to observe any division, either mitotic or amitotic in the parenchymal cells (p. 323), since, so far as can be determined from his statements, he has apparently paid little or no attention to the neck-region. Mere observation of the method of growth and the abundance of nuclei in this region forces us to conclude, either that nuclear divisions occur in enormous numbers in this region or else that nuclei are formed *de novo*, an alternative which most of us would hesitate to accept without proof of the most conclusive character.

When one examines scores of these neck-regions, as I have done, and finds first absolutely no mitoses and second hundreds of cases of apparent amitosis, many of them almost diagrammatic, the evidence for the occurrence of amitosis acquires at least some value.

On p. 322 Richards says: "Child has assumed all through his work that the absence of mitotic figures in tissues known to be growing rapidly is evidence of the occurrence of division by amitosis." He then proceeds to show that the growth of the parenchyma occurs to a large extent by the formation of "intercellular material." This statement of Richards seems, so far as it concerns my own position, to be an error. My argument is actually as follows: When mitosis is absent from regions where rapid increase in the number of nuclei is manifestly taking place, we have good reason to believe that some other form of nuclear division is occurring, especially when we have discovered what appears to be this other form in other tissues of the same animal.

I have, I think, made it sufficiently clear all through my work that in speaking of regions of rapid growth I meant not merely regions in which the cells were increasing in size or forming intercellular material, but those in which they were actually increas-

ing in number. I have never, however, considered absence of mitosis in such cases as proof of the occurrence of amitosis. Only the actual observation of amitosis can prove that it is occurring.

I wish to add to my earlier observations the fact that in a single individual consisting of scolex, neck-region and a few of the youngest proglottids, I have found almost every nucleus in mitotic division. This is the only case of the sort which I have ever observed. Aside from this I have never seen a single case of mitosis in this region of the body. I am inclined to believe that this is a young animal in the early stages of growth. In my work on various other forms, as yet unpublished, I have found considerable evidence in support of the view that amitosis becomes more frequent as growth (with cell division) becomes more rapid. In *Planaria*, for example, regeneration begins with mitosis, but as the growth of the new tissue becomes more rapid amitosis takes the place of mitosis. Patterson[1] has found that the frequency of apparent amitosis increases in regions of rapid growth, and other evidence to be presented elsewhere exists.

For those to whom the positive observations on amitosis are of little or no value as evidence this case of mitosis in the neck-region of *Moniezia* opens a way for simple interpretation. They will conclude, as Richards suggests (p. 320), that mitosis is of short duration and occurs in waves, *i. e.*, is periodic, and that all the other individuals observed by me are simply stages between two such periods. At present, however, I cannot accept such an interpretation as the correct one. Not only the cells of the neck-region, but the cells of the scolex, which does not take part in the growth of other regions are undergoing mitosis in this specimen. Moreover, I am as yet unable to convince myself that the many cases of apparent amitosis, which I have observed, in the neck-region of other individuals, are all errors of observation, or something else than nuclear division.

Concerning the structure of the nuclei in *Moniezia*, it may be said merely that the non-chromatic portions of the "embryonic nuclei" present widely different appearances with different fixing agents. After Hermann's fluid the nuclear structure of the primitive germ nuclei and the parenchyma nuclei is somewhat similar

[1] "Amitosis in the Pigeon's Egg," *Anat. Anz.*, XXXII., 5, 1908.

to that figured by Richards for *Tænia*, but after comparing the results of various fixing agents I came to regard it as highly probable that the "strands of linin" were simply coagulation products and therefore stated (I., p. 93) that "a distinct reticular structure does not appear." Richards (p. 316) attributes to me the statement that "the nuclei do not contain any definite reticulum." Though I am not desirous of splitting hairs this seems to me a much more positive statement than that which I did make. I do not know how widely different the nuclei of *Moniezia* are from those of *Tænia*, and while I could give figures showing "strands of linin" in some of these nuclei, I am strongly skeptical, for the reasons given above, as to their representing in *Moniezia* a real nuclear structure existing before fixation. As the germ cells approach maturation the visible nuclear structure undergoes alteration, as my figures show, and the non-chromatic portions show more definite and constant characteristics. Richards in his work has apparently employed only Flemming's and Zenker's fluids, both of which will, under certain conditions, produce reticular structure in solutions of proteids. Since the "strands of linin" were sometimes confusing in the study of the nuclear membrane I often found it desirable to extract the stain to such an extent that they were only slightly stained, or to employ fixing agents which did not show them in the resting nuclei.

But the chief interest centers of course about the occurrence or non-occurrence of amitosis in the germ cycle. It is evident that if all the nuclei which are included in a given proglottid arise, or may under certain conditions arise, in the neck-region by amitotic division the germ cells must arise from nuclei which have previously divided amitotically. This conclusion is, however, open to the objection that since we cannot be certain that every individual nucleus in the neck-region has divided amitotically, it may be possible that the nuclei of the germ cycle have not so divided. This objection is actually of little force, for there are probably not enough parenchymal nuclei in the neck-region at any one time to give rise even to all the mother germ cells which appear. Nevertheless this objection must be reckoned with and I should not for a moment maintain that the amitotic division in the neck-region constitutes absolute proof of the occurrence of amitosis in the germ cycle.

In my own work I proceeded posteriorly from the neck through the youngest proglottids to the region in which the reproductive organs first appear, and then followed their development from this point to and through the process of maturation. In the primitive germ cells mitosis is more or less frequent, as I have shown in my earlier papers, and if I had not found the most positive evidence of the occurrence of amitosis I should not have doubted that in this period cell division was wholly mitotic. But I found it is impossible to convince myself that this was the case, simply because I observed too frequently what I could not interpret as anything but amitosis.

Richards has not found amitosis in the germ cells, but he does not state what stages in the development of the reproductive organs he has examined. If one may judge from his figures and his statement on p. 313 that "the female sex cells in the cestodes in question are by far the largest cells in the body," it would appear that he has examined only the later stages, in which the cells have already assumed the definite characteristics of germ cells. In these stages I myself have never been able to observe amitosis with certainty, though mitosis is often seen.

It is during what one might designate as the embryonic development of the reproductive organs, when the germ cells, at least in the female, are not visibly different, from the cells which form the vitellaria and the reproductive ducts, that I have found amitosis, though even in these stages mitosis sometimes occurs. In order to forestall a possible objection, I should perhaps call attention again to the fact already noted in my earlier work (I., pp. 97–109) that after certain stages the portions which are to form the ovary can readily be distinguished from those which are to form vitellaria and ducts by their position and method of growth, though the cells are all still apparently similar.

Richards says absolutely nothing concerning these earlier stages in his work on *Tænia*, and until further evidence is forthcoming, it seems at least possible that his failure to discover amitosis may have been due to the fact that he examined only stages where it does not occur. However, I know nothing from personal observation as to the occurrence or non-occurrence of amitosis in the species which Richards has examined, and since

I am inclined to believe that the relative frequency of mitosis and amitosis in certain species, and even in single individuals, *e. g.*, *Planaria* may vary greatly according to conditions, it is also possible that Richards' material differs more or less widely from mine.

And now a few words concerning Richards' attempts at interpretation of my observations on the germ cells. On p. 315 he suggests that I have been misled by mistaking for a nucleus or part of a nucleus a mass of "Nebendotter" which occurs in "some of the early oögonia . . . while it is not present in some oöcytes" (p. 313). That one investigator working with one species should be willing, without more positive evidence than Richards has anywhere brought forward, to attribute to another, of wider experience than himself, and working with a different species and genus, so gross an error of observation as this is, to say the least, somewhat surprising. But the facts are these: as I have noted above, amitosis occurs in the earlier stages of development of the ovary and in such stages the primitive germ cells certainly contain no "Nebendotter." As a matter of fact, I have never seen anything of the sort at any stage in the ova of *Moniezia*, although the cells of the vitellaria, which in certain stages may easily be mistaken for ova, develop such masses rather early in their history, but usually not until division has ceased (see Child, I., Figs. 31–35, and pp. 112–3). I cannot see that my accounts of nuclear structure and division afford any possible basis for the relief that I could have mistaken for a nucleus a body which according to Richards stains readily with iron hæmatoxylin and appears "as a dark homogeneous mass even after a great deal of extraction," by which "nucleus and cytoplasm may be entirely decolorized" (Richards, p. 314). Moreover I have employed various methods of staining which leave the yolk almost wholly unstained.

Concerning the slight difference in color between the two parts of a nucleus, which I noted occasionally (Child, I., p. 95), it need only be said that I have often observed such differences in the somatic cells of *Moniezia*, as well as in the somatic cells of other forms, where there can be no question concerning "Nebendotter," consequently Richards' suggestion (p. 315) that

one of the parts in such cases is "Nebendotter" falls to the ground.

As regards Richards' interpretation of "endogenous" division (Child, I., p. 95), which he presents in connection with the case of mitosis shown in his Figs. 17 and 18, it may be said that such attempts at interpretation of another's observations are scarcely worthy of criticism. The statement of an author that he has employed so far as possible every precaution and has repeated his observations again and again to avoid error, is ordinarily given some degree of consideration. Moreover, as regards this particular point I stated (Child, I., pp. 95–6) that "some cases of this sort of almost diagrammatic clearness have been observed." And finally reference to a few figures (*e. g.*, I., Fig. 13*E*; II., Figs. 9*A* and 9*C*) is sufficient to show that the parts in such divisions are often very different in size and the membranes perfectly distinct throughout. There is then not the slightest basis for regarding them as misinterpreted mitoses.

To sum up: Richards' evidence for the non-occurrence of amitosis is wholly negative, my evidence for its occurrence is positive. How far his failure to find amitosis may be due to failure to examine the proper stages and how far to the actual absence of such phenomena as I have described, it is impossible to determine. Second, his attempts at interpretation of my results as errors of observation are wholly unsuccessful, since they concern stages of development very different from those on which I have based my conclusions and occurring in another genus, and since they involve a total disregard of much that I have said concerning methods, precautions, the appearance of the nuclei, etc.

The whole question of the occurrence or non-occurrence of amitosis is one which requires great care and extended research, and it may be doubted whether it is a suitable subject for a beginner in cytological investigation, both because of his lack of experience and because he is likely to be guided to a greater or less extent by the views of his instructor.

I wish to add a brief remark concerning my later observations on amitosis in other forms.[1] In this paper I merely recorded the

[1] Child, "Amitosis as a Factor in Normal and Regulatory Growth," *Anat. Anz.*, XXX., 11 and 12, 1907.

occurrence of what seemed to me to be undoubted cases of amitosis in a number of species from different groups of the animal kingdom, without attempting in most cases to determine its frequency or significance, and added a brief consideration of the physiological and theoretical significance of amitosis in general. Concerning my observations in this paper Richards says (p. 311) that my "evidence is lacking in some respects." I scarcely see how other evidence than positive statements and figures can be given and must suggest the desirability of more definite criticism than this, for in discrediting another's observations it is no more than just to present definite reasons for one's position.

And finally: if my observations are not in accord with certain current hypotheses it is not because of any preconceived opinions on my part concerning those hypotheses, but simply because the results of my work have forced me to believe that the hypotheses are not in accord with all the facts. As regards this point, it would seem that our present knowledge of regulatory phenomena in organisms is sufficient to show very clearly that certain results may be attained in different ways under different conditions. Moreover, the periodical recurrence of specific structures in definite form and number is one of the most characteristic features of the organic cycle, but we do not regard it as necessary to believe that those structures should persist as distinct and continuous entities during the periods when they are not visible. To speak specifically, why should we regard the chromosome problem as fundamentally different from the problem involved in the recurrence in each generation of five fingers upon a hand, or the regulatory development of a definite and characteristic number of tentacles in a piece of an actinian body? The farther we proceed in our physiological analysis of developmental phenomena, the more evident does it become that the finger and the tentacle do not persist as distinct and definite entities during all the period when they are not present as visible structures. The only thing which persists is the physiological capacity to react in a certain way under certain conditions, and when these conditions arise the characteristic structure-complex appears.

In short, when we consider the chromosome problem from a physiological instead of a purely morphological point of view, and

we must consider it in this way sooner or later, we can find no good reason for regarding it as fundamentally different from many other problems of ontogeny. Physiologically it is no more difficult to conceive that a piece of a nucleus should, under certain conditions, give rise to the characteristic number of chromosomes, than that a piece of the actinian body should give rise to the characteristic number of tentacles. The correctness of the chromosome hypothesis is far from being demonstrated, and in view of this fact we must consider the possibility of revising the hypothesis to fit the facts, as well as that of bringing the facts nto accord with the hypothesis.

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THE MANIFESTATION OF THE FLIGHT-FUNCTION IN THE SILKWORM (BOMBYX MORI).

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The activities of the adult silkworm moth (*Bombyx mori*) are normally confined to those connected with the reproductive reflexes, mating and ovipositing.[1] The moth issues from its cocoon sexually mature and lives in a perfectly functional condition for several days without taking food and scarcely wandering from its birthplace. Its thoroughly domesticated condition and associated habits call for the exercise of but few of the usual insect instincts. In particular, searching for food and for mates having been dispensed with, the flight-function is a useless function and rarely manifest.

In general appearance, the newly emerged moth is a perfectly formed insect. A close inspection, however, shows vestigial mouth-parts, the taking of food having thereby become an impossibility. Otherwise, the various anatomical features are normal. The wings, particularly, are of perfect form and under perfect control so that the exercise of the flight-function is a possibility, though as stated the insects are rarely observed to fly.

"The presence of an organ," says Folsom,[2] "normally implies ability to use it. The newly born butterfly needs no practice preliminary to flight." The silkworm appears, from general observations, to be an exception to this rule. At any rate the non-flight habit has seemingly become fixed, absolutely so in the case of the female, but in the case of the male with exceptions and under special conditions to be herein noted.

This non-flight habit is such a common observation among silkworm breeders that investigators have made use of the wing of the silkworm moth to study indications of its structural degen-

[1] Kellogg, "Some Silkworm Moth Reflexes," BIOL. BULL., V., pp. 152–154. McCracken, "The Egg-laying Apparatus in the Silkworm as a Reflex Apparatus," *Jour. of Comp. Neur. and Psychol.*, XVII., pp. 262–285, 1907.

[2] Folsom, "Entomology," p. 357.

eration on the basis of its being "functionally degenerate,"[1] and psychologists allude to the non-flight habit as indicating a loss of the "flying instinct."[2]

Especial interest therefore attaches to the fact that this apparently useless organ persists in a perfect form and under perfect muscular control.

Though slightly variable in the character of its venation, as pointed out by Kellogg and Bell, we do not know that the veins vary more than in the wings of moths of other species, and in comparing the wings of a long series of "non-fliers" with those of "fliers" I find no structural differences between the two lots. The wings appear, however, to be fundamentally non-useful, since both effectual mating and effectual ovipositing, the only adult activities, can be successfully accomplished without them. The de-winged silk-worm moth exhibits no inconvenience in these respects. Mayer-Soule[3] found that in certain species males deprived of wings met with greater resistance from females when the females were deprived of sight. The female silkworm moth, however, exercises no selection between the normal and de-winged male, nor does the male between normal and de-winged female.

Although the wings are normally not used in flight, as pointed out, they are habitually fluttered preliminary to mating. This fluttering of the wings may effect a circulation of the air in the neighborhood of the moths so that the presence of the female with extended scent glands is more readily discerned by the male. However, the natural proximity of males and females under the usual conditions of silkworm breeding renders this use of but little importance.

The wings are also made use of in the "turning reflex." A moth placed upon its back instantaneously rights itself by bringing the forewings quickly together and thus exerting a pressure upon the surface. This procedure follows in the decapitated as well as in the normal moth, and though one rarely necessary under usual conditions it shows the perfect muscular and nervous control under which the wings are held.

[1] Kellogg and Bell, "Inheritance in Silkworms," I., Stanford Univ. Pub., No. 1, 1908.

[2] Oppenheim, "Mentel Growth and Control," 1902, p. 99.

[3] Mayer-Soule, "Some Reactions of Caterpillars and Moths," *Jour. Exper. Zoöl.*, Vol. III., 1900.

Since, therefore, the wings are present and are under perfect muscular and nervous control, what is apparently missing in the normal moth is the flight "instinct," or the disposition to set in motion those reflexes that make for flight.

A study of the habits of the male silkworm moth has been made, therefore, with special reference to the flight function and some interesting suggestive conditions have been observed.

As previously stated, the mating instinct is strong in the young male. It is at once aroused if a female is near and the numerous reflexes that effect mating are initiated. Upon leaving its cocoon, under ordinary conditions, the male seeks the near-by female. After several hours in copula separation takes place. The female then begins to oviposit, and the male seeks another female and thus passes from female to female throughout the few days of its existence. Thus we may say that the mating instinct once aroused is the dominating and controlling instinct. In the absence of the female the male behaves somewhat differently. If a young male moth is isolated in a closed box and in a room with no females present, it remains for several days in a perfectly passive condition, scarcely moving. On the fourth or fifth day the moth begins to flutter wildly about within the box, and upon removal of the cover soars into the air, circling about and sustaining itself upon the wing for from thirty seconds to several minutes. It comes to rest seemingly from fatigue and may, after a few seconds, during which the wings are vigorously fluttering, again take flight for a few minutes finally coming to rest again to remain wherever chance may find it until death ensues.

Having by chance observed this behavior in several male moths, I selected, at the height of the breeding season, a large number of males as they issued from their cocoons and placed them in a large well-lighted room by themselves, some individually within closed boxes and others exposed each by itself on a small tray. In many cases the moth remained perfectly quiet for several days, then wandered about with a fluttering of the wings, finally coming to rest and remaining in a quiet condition until death. By far the greater number, about seventy-five out of one hundred, behaved as in the cases previously noted. They fluttered about vigorously on the fourth to the eighth days and then soared upward.

Thus it was ascertained that many male moths were not powerless with respect to flight. The moth has not "forgotten" how to fly. It would appear, however, that, during the first few days of the life of the moth the flight reflexes are under inhibition and remain so during the exercise of the mating reflexes. Restrained from mating, however, as has been demonstrated, the flight instinct asserts itself, and the flight reflexes are "let loose" as it were. Neither natural selection, which by carrying a "flier" far from a mate, and thus leaving it without offspring, nor the discontinuance of the need for flight have served to eradicate the possibility of exercising the flight function.

As stated, the moths thus far experimented with were deprived of mates. Later, a large series of moths was isolated after mating had taken place. They were first allowed to mate normally. When they had voluntarily left the female they were isolated as before. The result was the same. When first isolated they were quiet. After several days had elapsed a large percentage entered into the "fluttering" condition and flight ensued.

During the very beginning of the "fluttering" condition if a female is brought near, the male is attracted to her and mating takes place. If, however, the fluttering condition is well under way the male pays no attention to the female but flutters past her or over her, it may be, and finally takes to wing.

It would seem, therefore, from the foregoing observations that the flight reflex is, at the time the insect leaves its cocoon, part of the moth's sum-total of inheritance but is merely under inhibition.

The mating instinct is, however, exercisable at once, that is, is immediately dominant. A sufficiently long inhibition of the mating instinct appears, in a large number of individuals, to augment the flight reflex. Furthermore, with the flight reflex released, the mating reflexes are, for the time being, inhibited, or at least manifestation of the flight reflex takes place only during suspension of the activities of the mating instinct, and during the manifestation of the mating activity, cannot be aroused.

This manifestation is wholly, however, without purpose, is purely non-adapted. It is a manifestation apparently of what

Wheeler refers to as "latent heredity."[1] "The vestigial instinctive action," says Wheeler, "presents itself as an act of racial or phyletic recollection and must, like the representations of individual memory, depend on psychological dispositions abiding in latency, just as the visibly morphological characters of the adult organism arise from the visibly physiological dispositions in the germ plasm. These dispositions must be inherited with great tenacity and persistency, since vestiges, both instinctive and structural, often remain latent for generations and then suddenly manifest themselves under stress of extraordinary stimuli."

In the case at hand, the "extraordinary stimuli," whatever these may be, that produce the manifestation are not external. The movement has every appearance of being "spontaneous," the release of "internal energy." The behavior here noted is strikingly parallel to that noted by Jennings with reference to lower organisms. "Often, perhaps usually in lower organisms," observes Jennings, "movement in a certain direction is due only to the release of inhibition. The organism moves in a given direction because it is moving from internal impulse, and because movement in this direction is not prevented."[2] Jennings observes. further, that "the behavior of the lower organism at any moment depends upon its physiological state at that moment."

Flight in the silkworm, manifesting itself as it does, without external stimulus and when the activities of the mating reflexes are suspended, is apparently a spontaneous movement dependent conceivably upon the physiological condition of the insect for the time being, and involves the awakening of a latent instinct.

In the presence of the female, the whole attention of the insect is monopolized for the time being with the impetus to mate, This "state" supercedes every other possible state and prompts or guides the insect to one line of conduct only. The action which is the normal consequent of this state is mating; all other possible actions are suppressed, and under normal conditions, as stated, habitually suppressed. The manifestation of the flight function under these experimental conditions shows that, though

[1] Wheeler, "Vestigial Instincts in Insects and other Animals," *Amer. Jour. of Psychol.*, Vol. XIX., pp. 1–13.

[2] Jennings, "Behavior of Lower Organisms," p. 284.

this function is a part of the sum total of inheritance, the overwhelming development of the one instinct, that of mating, serves habitually to inhibit its activity. On the other hand, something has preserved both the wing structure and the flight function in spite of persistent non-use and natural selection, and although these have no relation to the preservation of the race in its present-day activities.

In the general conception of "instinct," its failure means extinction of the species.[1] In the case at hand, on the contrary its manifestation would mean extinction of the species. Its failure of manifestation is associated with the present-day life of the insect. Its manifestation under conditions herein observed attests the persistence in heredity of an ancestral function long after it has lost everything of a purposeful nature.

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[1] Jordan and Kellogg, "Evolution and Animal Life," p. 430.

A MATHEMATICAL TREATMENT OF SOME BIOLOGICAL PROBLEMS.

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Dr. King's work ('07, '09), "On Studies on Sex-determination in Amphibians," suggests an interesting problem which may be put in the following form:

A jar contains a large number of male and female tadpoles, the proportion of each being unknown: if on picking out $m + n$ tadpoles, m are found to be males and n females, to find the probability that the ratio of the number of either sex to the entire lot lies between given limits.

It will be seen that problems of this nature occur frequently in biological investigations and that it is of importance to have some method for determining the accuracy of the observed proportions. This can be done by means of the formula given below. As the development of the formula is somewhat complicated I shall present the entire process of the mathematical treatment of the solutions based on the theorem of Bayes, together with one application. Although such an elementary exposition of the subject will be superfluous for one who is familiar with the theory of probabilities nevertheless for others it may be helpful.

If p denote the probability that an event will happen, then $(1 - p)$ is the probability that the event will fail. If the probability that the event will fail on any single trial is $(1 - p)$, the probability that it will fail every time is $(1 - p)^n$. The probability that it will happen on the first trial and fail on the succeeding $n - 1$ trial is $p(1 - p)^{n-1}$. But the event is just as likely to happen on the second, third, etc., trials as on the first. Hence the probability that the event will happen just once in the n trials is

$$np(1 - p)^{n-1}.$$

Continuing this process, we can easily see that the probability that it will happen m times in $m + n$ trials is

$$\frac{\lfloor m+n}{\lfloor m \lfloor n} p^m(1-p)^n. \tag{1}$$

From (1) we can obtain directly the probability that exactly m males and n females occur in $m+n$ drawings and the expression will be

$$p = \frac{\lfloor m+n}{\lfloor m \lfloor n} x(1-x)^n \tag{2}$$

where x denotes males and $(1-x)$ females.

If we call the right hand member of (2) y, then

$$y = cx^m(1-x)^n$$

and this equation represents a curve with a zero ordinate when $x = 0$ and $x = 1$. Since the elementary area under the curve is

$$\frac{\lfloor m+n}{\lfloor m \lfloor n} x^m(1-x)^n dx,$$

the number of cases which occur between the two limits (a, b) is proportional to the sum of the differentials taken from a to b, or

$$\frac{\lfloor m+n}{\lfloor m \lfloor n} \int_a^b x^m(1-x)^n dx.$$

Also the totality of cases will be proportional to the sum of the differentials taken from 0 to 1, or

$$\frac{\lfloor m+n}{\lfloor m \lfloor n} \int_0^1 x^m(1-x)^n dx.$$

Hence the probability that the ratio of the males in the jar to the entire lot lies between the two limits is

$$p = \frac{\int_a^b x^m(1-x)^n dx}{\int_0^1 x^m(1-x)^n dx}. \tag{3}$$

This is the well-known theorem of Bayes.[1] This equation as it

[1] See Todhunter's "History of the Theory of Probability from the Time of Pascal to that of Laplace," 1865.

stands is applicable to cases where the number of observations is small. For cases where the number of observations is large we must modify this still further.

If we suppose the two limits to be $m/s \pm \theta$ where $s = m + n$, then equation (3) may be written in the following form :

$$p = \frac{\int_{m/s-\theta}^{m/s+\theta} x^m(1-x)^n dx}{\int_0^1 x^m(1-x)^n dx}.$$

By successive integration by parts the denominator is evaluated, giving

$$\int_0^1 x^m(1-x)^n dx = \frac{m!\,n!}{(m+n+1)!}. \tag{4}$$

If we let $x = m/s + z$ the numerator becomes

$$\int_{-\theta} \left(\frac{m}{s} + z\right)^m \left(\frac{n}{s} - z\right)^n dz,$$

which is approximately

$$\frac{m^m n^n}{s^s} \int_{-\theta}^{+\theta} \varepsilon^{-\frac{s^3 z^2}{2mn}} dz.$$

With the above transformation the formula becomes

$$p = \frac{(s+1)!}{m!\,n!} \frac{m^m n^n}{s^s} \int_{-\theta}^{+\theta} \varepsilon^{-\frac{s^3 z^2}{2mn}} dz. \tag{5}$$

Now if we apply Stirling's formula for large numbers (4) becomes

$$\frac{(s+1)!}{m!\,n!} = \frac{s^s}{m^m n^n} \sqrt{\frac{s^3}{2\pi mn}}.$$

Therefore (5) may be written in the following form :

$$p = \sqrt{\frac{s^3}{2\pi mn}} \int_{-\theta}^{+\theta} \varepsilon^{-\frac{s^3 z^2}{2mn}} dz.$$

If we assume

$$\sqrt{\frac{s^3}{2mn}}\, z = t$$

and substitute ω for

$$\theta\sqrt{\frac{s^3}{2mn}}$$

then as a final form we have

$$p = \frac{2}{\sqrt{\pi}}\int^{\omega} \varepsilon^{-t^2} dt.$$

Since the above expression is a well-known equation for the probability integral, the degree of probability for any given limits may be determined from the table. For computation we have the following relation :

$$\omega = \theta\sqrt{\frac{s^3}{2mn}} \quad \text{or} \quad \theta = \omega\sqrt{\frac{2mn}{s^3}},$$

and

$$p = \varphi(\omega).$$

As will be seen from the above relations, the degree of probability is proportional to the amount of deviation. Therefore in any given case we can either increase or decrease the value of probability by changing the value of the deviation. Thus in order to facilitate a comparison of several sets of data, it would be advantageous to fix the value of the probability and then determine the corresponding amount of the deviation. If we take 0.75 for the value of the probability, it will be certainly high enough for practical purposes. If we adopt this system, then the corresponding value of ω is fixed and is equal to 0.814. Thus we do not even need to use the table to determine the amount of deviation. If however one wishes to find any other value of the probability than I have proposed (*i. e.*, 0.75), such can be readily obtained from the table. Thus the determination of the deviation can be made by a simple arithmetical process.

The following will illustrate a method of determination. Dr. King has kindly supplied the data for this purpose and I wish to thank her for that material.

Example : Out of 16,100 tadpoles, 9,949 are examined. 5,136 are found to be females and 4,813 males. Find the probability that the ratio of females to the entire lot lies between given limits.

We have

$$\theta = \omega \sqrt{\frac{2mn}{s^3}}. \qquad p = \varphi(\omega) = 0.75.$$

Since we adopted the fixed value for probability (0.75) the value of ω is also fixed and equal to 0.814. Thus our problem is therefore to find a value of θ when the value of p is known.

$$\theta = 0.814 \sqrt{\frac{2 \times 4{,}813 \times 5{,}136}{99{,}493}} = 0.814 \times 0.00709 = 0.0058.$$

Deviation $= 16{,}100 \times 0.0058 = \pm 93$.

Total number of females $= 16{,}100 \times 5{,}136/9{,}949 = 8{,}311 \pm 93$.

With this value of probability we conclude that the total number of females in the entire lot is neither greater than 8,404 nor less than 8,218.

TEMPERATURE AS A FACTOR IN THE DETERMINATION OF SEX IN AMPHIBIANS.

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In 1905, Hertwig (1) published the results of a series of experiments on the eggs of *Rana temporaria* and of *Rana esculenta* which showed, according to his interpretation, that temperature is a sex-determining factor in amphibians: a high temperature favoring the development of females; a low temperature causing the production of relatively more males. This conclusion is in accord with the results of Issakówitsch's (2) earlier experiments on sex-determination in *Daphnia;* but it is opposed by the results of Maupas' (7) experiments on *Hydatina senta* and by those of von Malsen (6) on *Dinophilus apatris*. The latter experiments seemed to indicate that heat leads to the development of males and that cold tends to the production of females. Maupas and Hertwig believe that temperature acts directly on the sex-cells: Issakówitsch and von Malsen, on the other hand, maintain that nutrition is the dominant factor in sex-determination, temperature acting only indirectly through its influence on the processes of assimilation in the parent organism.

In the spring of 1907, I made a series of temperature experiments on the eggs of the toad, *Bufo lentiginosus*, which were carried out in a manner similar to those made by Hertwig on the eggs of *Rana*. Various lots of fertilized eggs were placed in tanks in which the water was kept at a nearly constant temperature until the tadpoles underwent metamorphosis (King, 5). In *Bufo* as in *Rana*, the sexes cannot be distinguished until the end of metamorphosis unless the gonads are sectioned. It is conceivable, therefore, that sex in these forms may not be determined until a relatively late period and that temperature, acting during the entire growth period of the tadpoles, might alter the normal sex-ratio. Two series of experiments were made with eggs from different females. In one series (lot A) 62.5 per cent. of the in-

dividuals in which sex was ascertained were females, although one half of the eggs had developed in water which had a temperature of 22–30° C. while the other half of the eggs had been kept at a temperature of 14–18° C. In the second series (lot B), the eggs were subjected to conditions similar to those under which the eggs of lot A had developed. Only 33.97 per cent. of females was obtained in this series, and more females were found among the individuals which had developed at a temperature of 14–18° C. than among those which had been kept at the higher temperature. The results of these experiments, therefore, do not support Hertwig's contention, and they seem to indicate that temperature, acting during the development of the tadpoles, does not determine sex in *Bufo*.

In the series of experiments described above the eggs belonging to lot A were fertilized in water which had a temperature of 16–18° C.; the eggs used for lot B, on the other hand, were fertilized in water which had a temperature of only 11–13° C. The results obtained in the experiments suggested the idea that temperature, acting at the time that the eggs were fertilized, might determine sex. This suggestion was tested in the spring of 1908 in the following way: a pair of toads were placed in water which was kept at a temperature of 26° C. until the eggs had been deposited and fertilized; the eggs from a second female were laid and normally fertilized in water which had a temperature of 9° C. There was thus a difference of 17° C. in the temperature of the water in which these two sets of eggs were fertilized. In each series as many individuals as possible were carried through to metamorphosis and their sex ascertained. The results obtained in these experiments are summarized in the following table.

TABLE I.

Water Temperature.	Number Sex Ascertained.	Males.	Females.	Per Cent. Females.
26° C.	2,178	953	1,225	56.24
9° C.	2,083	1,181	902	43.30

The average proportion of females among young toads that have recently completed their metamorphosis is 51.62 per cent., judging from the number of females found among the 9,949

individuals whose sex I have ascertained during the course of the experiments which I have made to study the problem of sex-determination in this form. No very definite conclusions can be drawn from the results of the experiments summarized in Table I. The percentage of females obtained from the lot of eggs that was fertilized at a temperature of 26° C., although higher than the probable average for the species, is still within the limits of possible normal variation in the proportion of females developing from eggs laid by different individuals. A relatively low percentage of females developed from the lot of eggs that was fertilized at 9° C., yet this percentage is about the same as that obtained in one of the series of experiments made to ascertain the influence of nutrition on the determination of sex in *Bufo* (King, 4). It may be possible to explain the results obtained in this last series of experiments, as well as those of the first series of temperature experiments briefly described above, as due to the fact that occasionally a batch of eggs gives an unusually low or an unusually high percentage of females and that by chance such exceptional lots of eggs were used for these experiments.

As this last series of experiments was carried out it is open to serious criticism. The eggs that were fertilized in warm water were laid by one female, while those that were fertilized in the cold water were laid by another individual. This gives an opportunity for the results to be influenced by possible normal variations in the sex-ratios of different lots of eggs. The two sets of eggs were fertilized by sperm from different males also and, as Morgan (8) has pointed out, there is the possibility that the male is responsible for the determination of sex in amphibians.

In order to avoid all obvious sources of error and, if possible, to obtain results that would give definite conclusions, another series of experiments was made this past spring with the eggs of *Bufo*. On April 7, 1909, a female which had just begun to lay was killed by pithing and the eggs removed to a dish of fresh water. Lots of about 350 eggs each were artificially fertilized in water which was kept at given temperatures (35° C., 30° C., 20° C., 10° C., 5° C.) for twenty minutes and then allowed to come gradually to the room temperature (18° C.). In all cases sperm from the same male was used. The extreme temperatures at

which lots of eggs were fertilized were 35° C. and 5° C. It is highly improbable that in a state of nature the eggs of *Bufo* are ever fertilized at these temperatures which are very injurious to the unsegmented egg, although they do not seriously interfere with the development of segmented eggs unless allowed to act for a comparatively long period of time (King, 3). Not more than one half of the eggs subjected to extreme temperatures were fertilized. Segmentation of these eggs did not begin until some three hours after the experiments were started, and in a majority of cases the cleavage planes came in very irregularly. A considerable number of eggs that began segmentation died within a few hours, so that but a very small number of individuals continued their development. After the eggs in these lots had reached the gastrulation stage they had apparently outlived the injurious effects of extreme temperature, as only three of the tadpoles that developed from the eggs that were fertilized in water with a temperature of 35° C. and but two from those that were fertilized at 5° C. died before it was possible to ascertain their sex. The great majority of eggs that were fertilized at medium temperatures, 30° C., 20° C. and 10° C., segmented normally and continued their development. The mortality in these lots was very low, not more than 6–8 per cent. of the individuals dying before it was possible to ascertain their sex. The various lots of tadpoles received similar food and were all kept as nearly as possible under similar external conditions during their entire growth period.

In this series of experiments, and also in several earlier ones, many of the tadpoles died after beginning their metamorphosis. In a considerable number of these individuals it was necessary to section the gonads in order that sex might be ascertained. The toads that survived metamorphosis were placed in large glass jars containing moist sod, and they were fed on small insects for several days. Young toads grow very rapidly, and by keeping them in the manner indicated for a short time it is possible to ascertain the sex of practically every individual without resorting to the tedious process of preserving and sectioning the gonads.

The results of this series of experiments are summarized in Table II.

TABLE II.

Water Temperature.	Number Sex Ascertained.	Males.	Females.	Per Cent. Females.
35° C.	90	42	48	53.33
30° C.	197	96	102	51.26
20° C.	323	157	166	51.39
10° C.	214	108	106	49.53
5° C.	64	33	31	48.43
Total	888	436	451	50.90

With a difference of 30° C. in the temperature of the water in which the two lots of eggs at the extremes of the series were fertilized there is a difference of only 4.95 per cent. in the proportion of females that developed in these lots. This difference is so small that it is evident that temperature, acting at the time of the fertilization of the eggs, is not the dominant factor in the determination of sex in *Bufo*.

In this series of experiments, with one exception, the proportion of females that was obtained in the different lots varied directly as the temperature at which the eggs were fertilized; relatively fewer females developing as the temperature of the water was lowered. The decrease in the percentage of females was so slight in the various cases, however, that I do not think it can have much significance, particularly as there were so few individuals in the lots at the extremes of the series. Had the number of individuals that metamorphosed been the same in every lot, practically the same sex-ratio would doubtless have been obtained for the entire series.

For the purpose of comparison, and to show more conclusively than is shown by the results of the series of experiments summarized in Table II. that temperature, acting at the time of the fertilization of the egg, does not determine sex in *Bufo*, the results obtained in former experiments in which different batches of eggs were laid in water with a known temperature are brought together in Table III.

TABLE III.

Water Temperature.	Number Sex Ascertained.	Males.	Females.	Per Cent. Females.
26° C.	2,178	953	1,125	56.24
16–18° C.	232	87	145	62.50
11–13° C.	156	103	53	33.97
9° C.	2,083	1,181	902	43.30

A comparison of the figures given in Table II. with those of Table III. shows that there is not the uniformity in the results of these various series of experiments that one would expect to find if temperature alone determined sex at the time of the fertilization of the egg. A lot of eggs that was fertilized at a temperature of 26° C. gave a higher percentage of females than was obtained from a lot that was fertilized at a temperature of 35° C., while the highest percentage of females obtained as yet in any experiment (62.5 per cent.) was found in a lot of individuals that developed from eggs that were fertilized at a temperature of 16–18° C. Of the various series of eggs that were fertilized in cold water, those that were fertilized at 9° C. gave a much lower percentage of females than did other lots that were fertilized at 10° C. or at 5° C. Although these results show very conclusively that temperature, acting at the time of the fertilization of the egg, is not the dominant factor in the determination of sex in *Bufo*, they do not exclude the possibility that temperature may have an indirect action on the determination of sex in this form. In every series of experiments lots of eggs that were fertilized at a temperature of 13° C. or below have given a lower percentage of females than has been obtained in the individuals developing from lots of eggs that were fertilized at higher temperatures. It is conceivable that a low temperature might act more injuriously on the female-producing spermatozoa than on those that are male-producing, if it be that there is a dimorphism in the spermatozoa of *Bufo* and that the male determines sex. This would, of course, greatly increase the chances that an egg laid in cold water would be fertilized by a male-producing spermatozoön. If, on the other hand, sex is determined in the egg, it is possible that the sex-determining mechanism is so evenly adjusted that temperature, under certain conditions, may turn the scale in one direction or the other.

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DIFFERENTIATION IN HYDROID COLONIES. II. AGLAOPHENIA.

H. B. TORREY.

In a former paper[1] I called attention to the fact that many species of hydroids change their structural type with age, that is, in the direction of their growth. "Stems straight proximally may become sinuous distally. Branches which alternate during the early stages of colonial development may later originate in pairs. Length and annulation of hydrothecal pedicels, size, proportions and ornamentation of hydrothecæ may similarly vary with the distance from the base of stem or branch." An attempt to analyze the problems in differentiation suggested by these facts was begun, upon a species of campanularian peculiarly well adapted for experimentation. The results reached favored the view that the progressive changes in the differentiation of the individuals of a colony are not dependent upon changes of external conditions, but upon changes of internal conditions; and that these changes are of a physiological character, and do not lead to a conception of *morphological determinants* or *residual germ plasm.*

The bearing of the facts upon the problem of senescence was also suggested. For, on the stem of such a hydroid as *Campanularia* — with a cymose type of budding — the individuals appear as successive generations, forming, at the same time, parts of the whole colony. Accordingly, a comparison of any two individuals might be expected to show a difference in their structure corresponding in some degree to a period of physiological change in the colony equal to their difference in age.

Since the publication of these results, several important papers have appeared expressing radically different views on the relation of natural death and development. Minot (*Pop. Sc. Mo.*, Nov., 1907, p. 472) holds that as cells differentiate they lose something of their capacity to live. Loeb (*Pfluger's Arch.*, 124, 1908, p. 411), after stating this conclusion in chemical terms as follows:

[1] Univ. Cal. Publ., Zoölogy, 2, p. 323.

that the chemical causes of death are identical with the chemical processes that lie at the basis of development itself, differs with it radically. He asserts, as a result of experiments with the eggs of sea urchins, that natural death and development are determined by chemical processes that have absolutely nothing to do with each other.

My own investigations, impeded, among other causes, by scarcity of material, have not yet reached a definite conclusion in this direction, although the results so far obtained are not inconsistent with the results of Loeb's brilliant experiments. Meanwhile, the facts as they appear in seven species of *Aglaophenia*, may be of some service to experimenters interested in the general problem.

As is very well known, a typical colony of the plumularian hydroid *Aglaophenia* closely resembles a feather, of which the shaft is represented by the stem and the vanes by two ranks of alternating branchlets, or hydrocladia, corresponding to barbs. Each hydrocladium is divided by more or less definite nodes into internodes and bears on one aspect — the same in all hydrocladia — a compact series of hydranths, one to each internode, with tooth-rimmed hydrothecæ. Associated with each hydrotheca are three tubular nematophores, a pair laterally placed, to the rear, and one in front in the median line (mesial).

Inspecting such a colony for signs of the differentiation connected so clearly with age in *C. bakeri*[1] one would naturally look for differences associated, first, with the growth of the colony as a whole, second, with the growth of individual hydrocladia. The former might be determined by a comparison of corresponding internodes of different hydrocladia, the latter by a comparison of successive internodes of the same hydrocladium.

In both cases, this procedure has established a correlation between differentiation and growth.[2] Since the picture of the correlation is not equally clear and elaborate in all species studied, I will describe it first for one of the most satisfactory in these respects, namely, *A. octocarpa*.

[1] *Loc. cit.*

[2] See Pearl, Publ. Carnegie Inst. 58, 1907, and, more recently, Ritter, Univ. Cal. Publ. Zoöl., 6, 1909, p. 64.

It should be understood, in examining all the tables and figures which follow in this paper, that they make no pretense to be

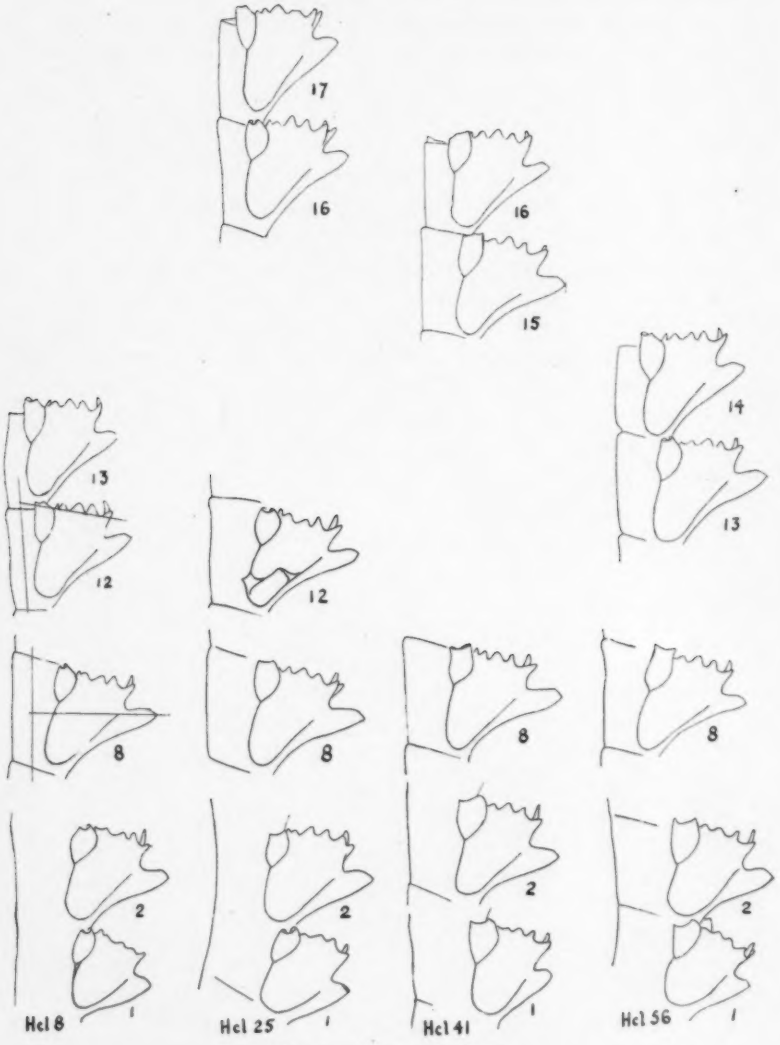

FIG. 1. *A. octocarpa*, × 45.

statistically thorough. They are rather illustrative of certain facts of differentiation that do exist and are capable of experimental analysis.

A. octocarpa. — A glance at the accompanying camera drawings (Fig. 1) will acquaint one with the main differentiating characters on which the present study is based. The four series of figures represent hydrothecæ from four hydrocladia. That they might be representative of the colony, the latter were selected at various levels on the right of the stem, being nos. 8, 25, 41 and 56, in a series of 64,[1] and care was taken that they should be the longest obtainable and, if possible, not damaged at their distal ends. Similarly, the hydrothecæ, drawn in profile, were chosen as representative of the whole hydrocladium, being nos. 1, 2, 8, usually 12, and the terminal two.

In the condensed picture of the conditions throughout the colony thus obtained, certain facts stand out prominently:

1. Each hydrocladium tapers from base to tip.[2]

2. On each hydrocladium, hydrotheca no. 1 is noticeably smaller than the others, and the mesial nematophore associated with it is especially short.

3. From base to tip, the openings of the hydrothecæ become less oblique to the axis of the hydrocladium. This obliqueness may be shown numerically by measuring the angle made with the axis of the internode by a line drawn tangent to the depressions between the first and second, and the third and fourth, lateral marginal teeth on each hydrotheca (12, *Hcl. 8*, Fig. 1). Two series of measurements from different colonies are shown in Tables I. and II.

4. The mesial nematophore reaches its greatest prominence toward the middle of the latter and then declines to the tip. This fact is numerically expressed in Table III., which shows the profile width of each hydrotheca to the point of the mesial nematophore on a line perpendicular to the axis of the internode (see Fig. 1, *hydrocl. 8*, *hth. 8*).

[1] The tip of the colony was wanting.

[2] This, as shown by repeated observations, is independent of the thickening of the perisarc with age. For convenience, neither such thickenings of the walls nor the septal and intrathecal ridges characteristic of the species have been drawn, except in 12, *Hcl. 25*, where the intrathecal ridge is shown.

TABLE I.

Measurements in Degrees of Angle.

Hydrocladium.	Hydrotheca.								
	1	2	8	12	13	14	15	16	17
8	114	110.3	108.2	103	96				
25	103.8	99.3	108	102.8				100	96
41	115.2	109	105				108	99	
56	116.2	116.5	105.5		99	91.2			

TABLE II.

Hydrocladium.	Hydrotheca.				
	1	2	7	11	
1	122	111.5	101.5	89.7	(12 hydrothecæ present)
40	99.7	111.8	101.5	100	(14 " ")
85	111.3	111.2	103.7	100.5	(13 " ")

TABLE III.

Measurements in mm. × 95.

Hydrocladium.	Hydrotheca.								
	1	2	8	12	13	14	15	16	17
8	24	30.5	32	27	25.5				
25	25	32	34	31				29.5	27.5
41	23.5	30	33.5				31.5	29	
56	25.5	30.5	30.5		30.5	27			

The facts formulated above concern the differentiation of internodes of single hydrocladia from various regions of the colony. On further examination, Tables I. and III. suggest that the character under consideration tends to decline from its maximum less rapidly on hydrocladia in the middle region of the colony than on hydrocladia either proximal or distal to them. This suggestion that the colony may have a typical form, exhibiting a differentiation from base to tip, analogous to what is so familiar in the leaves of plants, is supported by series of measurements at the tips of several colonies. One such series is presented in Table IV., in which are given both the length of each theca, through the unpaired or mesial tooth (upper figures), and the width of the theca through the tip of the mesial nematophore (lower figures in italics), as in Table III.

TABLE IV.

Measurements in mm. × 95.

Hydrocladium.	Hydrotheca.																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
3 (from tip)	—	36.5	38	38													
	27	*32*	*32*	*31*													
4 " "	—	37	37.5	37	37												
	—	*33*	*34.5*	*33*	*31*												
7 " "	—	37	—	38.5	38.5	38	38	—	38	36							
	—	*33*	—	*36*	*35*	*35*	*34*	—	*33*	*31*							
10 " "	—	—	37	—	38	—	38	—	—	38.5	—	38	38				
	—	—	*34*	—	*35*	—	*35*	—	—	*35*	—	*31.5*	*28*				
13 " "	32	35.5	38	38	38.5	—	37	—	38	38	38	38	37.5	38.5	37	38	—
	25±	*34*	*35*	*35*	*34*	—	*35*	—	*35*	*35*	*34.5*	*34.5*	*34.5*	*34*	*33*	*30.5*	—

It will be seen that, as the tip of the colony is approached, not only do the hydrocladia possess fewer and fewer hydrothecæ, but the dimension of the latter through the mesial nematophore reaches its minimum more and more rapidly. Since the hydrothecæ, once formed, do not enlarge with age, it is clear that for such colonies as this, there is a limit of growth and a specific form.[1]

A. pluma. — This species not only supports the statements made for *A. octocarpa*, though somewhat less conspicuously, but presents another character (of no value in *A. octocarpa*) by which differentiation in the hydrocladia may be determined, namely, the length of the hydrotheca through the mesial tooth. Fig. 2 presents graphically, in concentrated form, the characters of a single colony so far as they concern the discussion. The measurements taken from the drawings are represented in the accompanying tables. It should be said, in this connection, that these measurements are subject to a certain amount of correction, owing to the great difficulty experienced at times in obtaining true profiles of the hydrothecæ. In hydrocladium no. 25, for instance, the hydrothecæ have suffered a degree of rotation which could not be rectified by the means at my disposal. The application of pressure sometimes suffices, but is very liable to produce more serious distortions. Fortunately, the errors which

[1] According to Table IV., the length of the hydrothecæ does not appear to be a significant dimension in *A. octocarpa*.

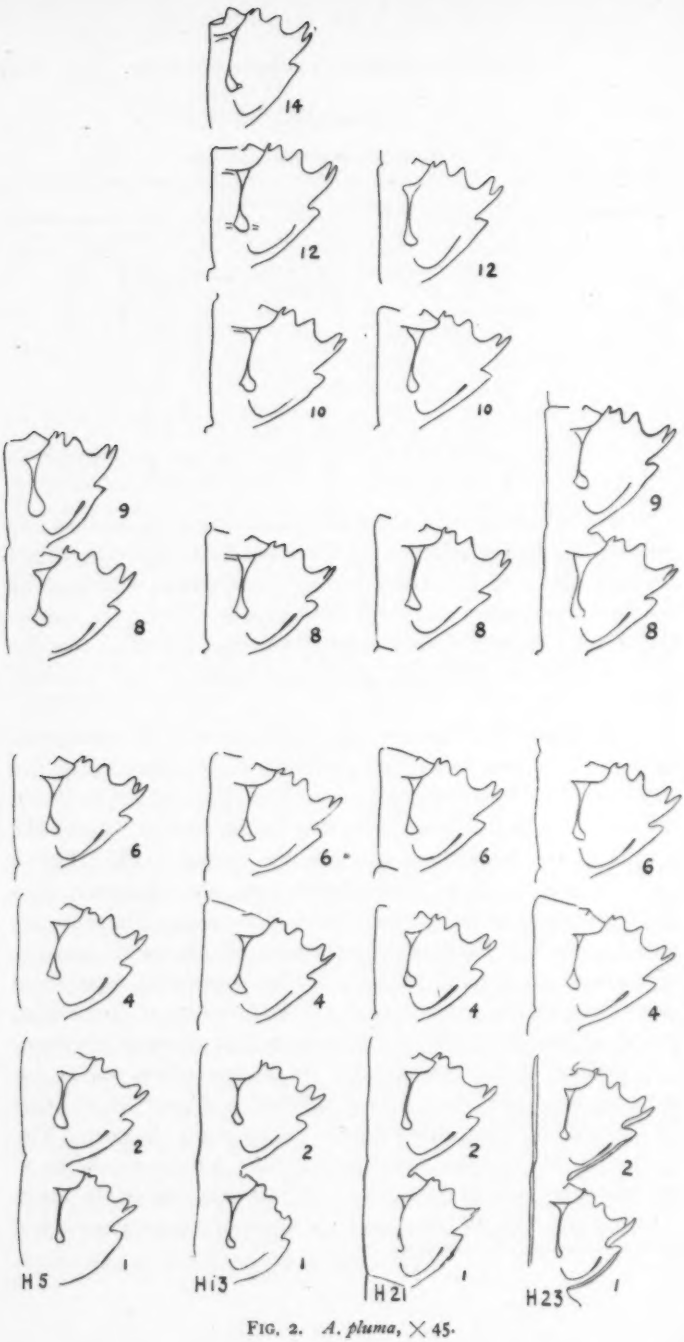

FIG. 2. *A. pluma*, × 45.

thus appear to be unavoidable, do not disturb in any essential respect the conclusions based on the measurements.

Differentiation within the several hydrocladia will first be considered.

In Table V., appear the measurements of the length of the mesial nematophore along a line perpendicular to the axis of the internode (upper figures) and the width of the hydrotheca to the tip of the nematophore along the same line (lower figures). The width of the hydrotheca along the line chosen varies, as shown by the table, independently of the length of the nematophore along the same line. Neither character is of much value

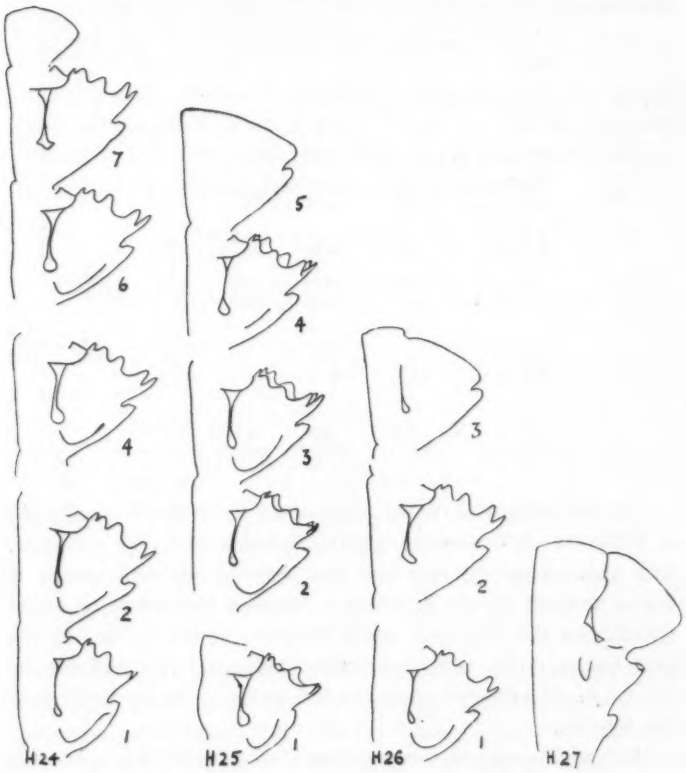

FIG. 2

to the experimenter in this species, owing largely to the slight degree to which the nematophore projects, and the narrow range of its variation in length, in fractions of a millimeter, as compared with the fluctuations in width of the hydrothecæ. There is no doubt, however, that the nematophore tends to vary, as in *A. octocarpa*, with the growth of the hydrocladium, its maximum length lying in the middle region of the latter.

TABLE V.

Measurements in mm. × 95.

Hydrocladium.	Hydrotheca.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
27	bud													
26	3.3 21.3	3.5 25	bud											
25	—	*4.5* 23.5	4 24.5	4 23	bud									
24	3.4 19.3	4.2 23.5		*4.6* 24.5		4 25.5	3.8 24.5	bud						
23	3.8 19.5	3.8 22		*4.5* 25.5		*4.4* 26.5		3.5 24.5	3.8 24.5	bud				
21	3.8 19.7	3.2 23		3.5 23		*4* 23.2		3.5 24.5		3 24.5		3.8 24.5	bud	
13	2.7 18.5	3 21.5		3 23.5		3.5 24		*3.9* 24		3.6 23.5		3.2 24.5		2.8 21.5
5	3.3 19.5	4 22.5		3.5 24		*4.3* 25		4 25	3 21.5					

The obliqueness of the openings of the hydrothecæ is recorded in Table VI., in degrees of angular distance, as for *A. octocarpa*, with this exception, that the line defining the obliqueness is drawn tangent to the depressions between the mesial and first lateral, and the third and fourth laterals (the margin having but nine teeth). This is a more stable standard, but could not be utilized in *A. octocarpa*, owing to the shape of the hydrotheca in that species.

With slight irregularities, the chief among them being connected with the variability of hydrotheca no. 1, the obliqueness diminishes toward the distal end of each hydrocladium.

TABLE VI.

Measurements in Degrees of Angle.

Hydrocladium.	Hydrotheca.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
27	bud													
26	128	125.5	bud											
25	128	131.5	125.5	126	bud									
24	129.8	130		128.5		123.3	*119.3*	bud						
23	127.5	129.8		129.3		125.5		123.5	*119.3*	bud				
21	130.3	129		120		120		120		119		*115.3*	bud	
13	—	125		128.2		123		124		121.5		117		*115*
5	133	131		121.5		121.5		120.2	*115*					

The lengths of the hydrothecæ, taken through the mesial tooth, are recorded in Table VII. There is a definite tendency to increase in length toward the distal end of each hydrocladium, the longest hydrotheca, however, being subterminal.

TABLE VII.

Measurements in mm. × 95.

Hydrocladium.	Hydrotheca.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
27	bud													
26	30.5	?	bud											
25	27.5	31.5	*34.5*	?	bud									
24	30.5	33.5		34.2		*36.5*	bud							
23	28	30.5		34.5		36		35	*36.5*	bud				
21	31	33		32		33		35+		*36*		35	bud	
13	—	30		31.5		34.5		34		36		*37.3*		32.5
5	—	29.5		33		34.5		*35.5*	33.5					

In Table VIII., the widths of the hydrothecæ previously used have been taken along the line defining the obliqueness of the opening of the hydrothecæ. As the figures show, this character is too variable to be very useful to the experimeter. Nevertheless, in common with the characters considered in the three tables

TABLE VIII.

Measurements in mm. × 95.

Hydrocladium.	Hydrotheca.													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
27	bud													
26	20	*22*	bud											
25	18	18.7	*20.5*	20	bud									
24	17.5	21		*21.5*		21	20	bud						
23	19	21.2		21		*22*		21.5	21	bud				
21	17	20.5		20		21.5		*22.3*		21		22	bud	
13	—	20.5		20.5		20.5		20.5		21.2		*22.3*		20.5
5	17.5	20		20.2		*22*		*22*		19.7				

just preceding, it aids in establishing the fact that colonies of *A. pluma*, like colonies of *A. octocarpa*, have a limited growth and a specific form, the hydrocladia, with respect to given characters, differing in different regions. To appreciate this fact, it is only necessary to note the position (1) of the maxima on the various hydrocladia in Tables V. (upper figures), VII. and VIII., and (2) of the minima in Table VI., especially in connection with hydrocladia 5, 13, 21, 23 and 24.

A. inconspicua.[1]—This small species is represented most scantily in the collections of the University of California. No conclusions can be offered concerning the form of the colony as a whole, as observations on the tip of a mature colony are at present out of the question. From the drawings (Fig. 3) of the internodes from two hydrocladia, however, and the measurements based upon them, Tables IX. and X., the species possesses admirable characters for experimental work, in the prominence of the mesial nematophore and the obliqueness of the hydrothecal opening.

These figures are typical, similar measurements from other hydrocladia and other colonies giving results that vary, within the hydrocladium, in no essential respect from them. It may be said that the hydrotheca at the base of each hydrocladium is especially variable and often differs conspicuously from those distal to it in size, shape, marginal dentation, and position on the

[1] Torrey, Univ. Cal. Publ., Zoöl., 1, 1902; *ibid.*, 2, 1904.

hydrocladium (owing to a slight rotation out of the plane of the other hydrothecæ). Nothing sufficiently definite can be said about the length or thickness of the hydrothecæ. There is a perceptible increase in size of the hydrothecæ in *Hcl. 21*, toward the tip. It is much less in the other hydrocladium; and meas-

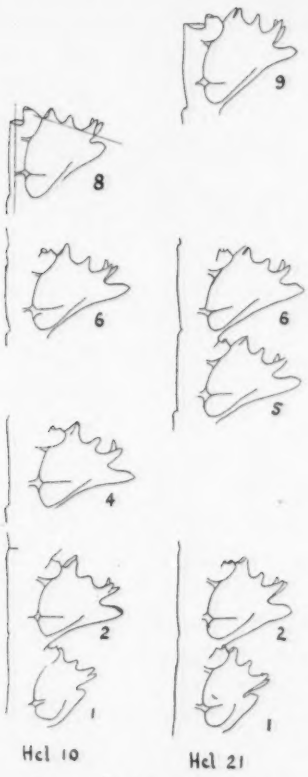

FIG. 3. *A. inconspicua*, × 45.

urements of others show that it is not characteristic and typical, for either dimension.

A. lophocarpa. — The few colonies of this species at hand are all imperfect, both as to stems and hydrocladia. However, the hydrocladia taper from base to tip, the first hydrotheca on each

TABLE IX.

COLONY WITH 28 HYDROCLADIA.

Measurements, in mm. × 95, of the profile width of each hydrotheca in Fig. 3 to the point of (1) the mesial nematophore on a line perpendicular to the axis of the internode (lower figures), and (2) the length of the mesial nematophore on the same line (upper figures).

Hydrocladium.	Hydrotheca.						
	1	2	4	5	6	8	9
10	2.5 16	7.3 26	8.2 29.5		8 24	4.8 24	
21	4.5 17	7 26		8.8 28.5	7.6 29		7.5 27.5

TABLE X.

Same colony, showing angles made with the axes of their internodes by the planes of the hydrothecal openings, the latter being represented in each case by a line tangent to the depressions between lateral marginal teeth 1 and 2, and 4 and 5 (see *Hcl. 10, hth. 8*).

Hydrocladium.	Hydrotheca.						
	1	2	4	5	6	8	9
10	125.5	121.3	122		114	105	
21	132.3	130		123.2	121		105.3

is smaller than the others, and there is a distinct tendency toward a reduction of the obliqueness of the hydrothecal opening, from base to tip. The mesial nematophore, also, projects farther from the hydrotheca in the intermediate internodes than at either extreme of the hydrocladia.

A. struthionides. — The obliqueness of the hydrothecal openings in each hydrocladium is practically constant in this species (Fig. 4). As in *A. octocarpa*, however, each hydrocladium tapers from base to tip, and the mesial nematophore is most prominent between the extremes. The prominence of the nematophore is, perhaps, of all the characters, most useful to the experimenter. The measurements in Table XI. show that the length of the nematophore along a line passing through its tip and perpendicular to the axis of the internode, varies independently of the width

of the hydrotheca along the same line from the base of the nematophore (see Fig. 4, *BHcl, hth.12*).

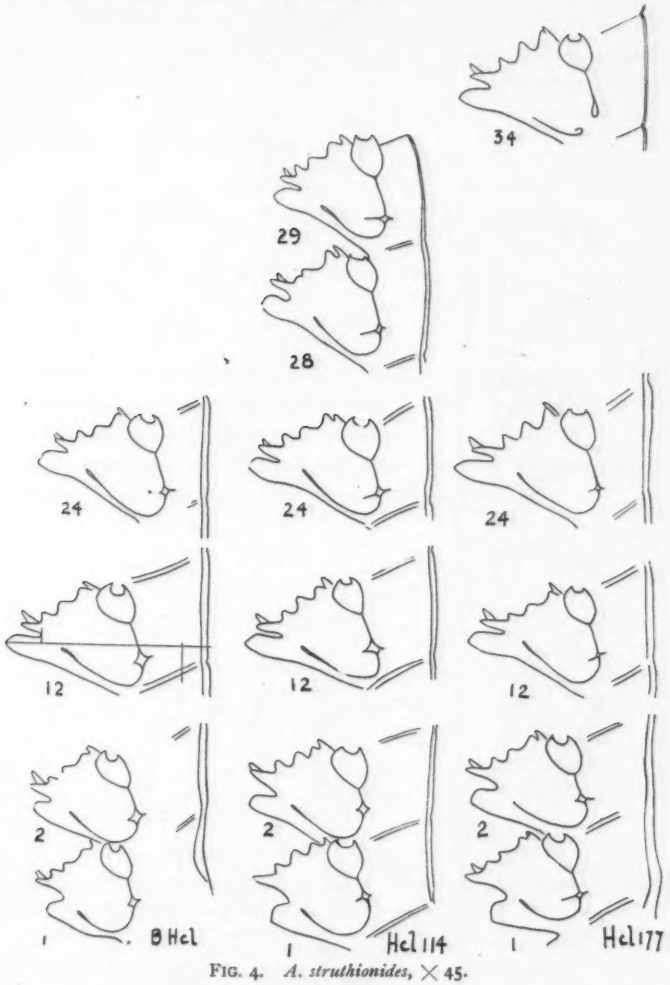

FIG. 4. *A. struthionides*, × 45.

Neither this nor the following species lends itself easily to a determination of colonial differentiation, as does *A. pluma*. Both are variable, often luxuriant growers. Colonies of *A.*

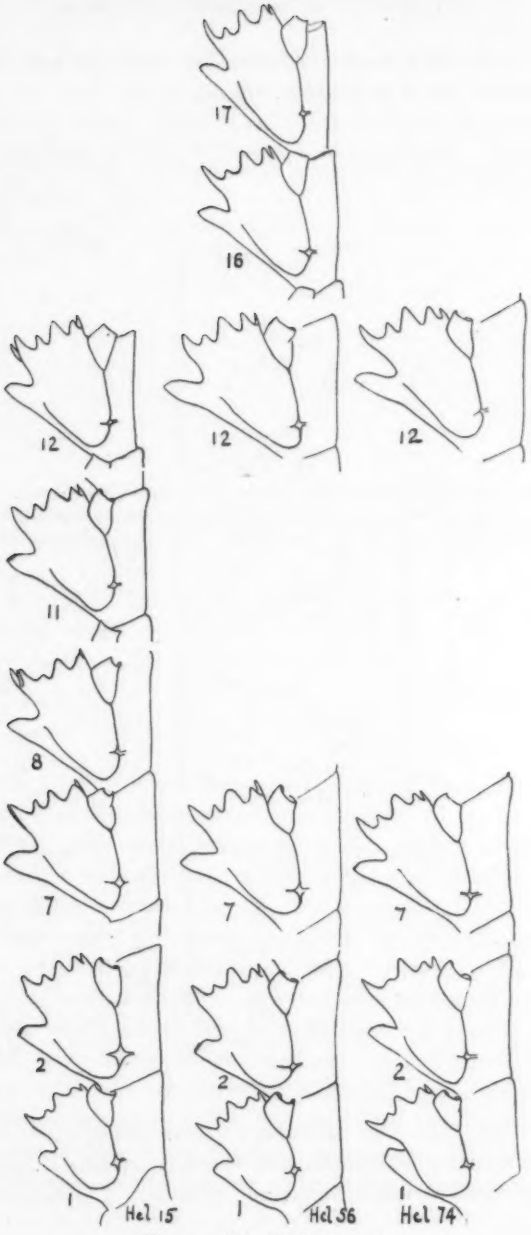

FIG. 5. *Aglaophenia* sp., × 45.

TABLE XI.

Measurements in mm. × 95.

		Hydrotheca.			
		2	12	24	34
Hcl. at base	1. Base of nem. to tip	5	11	9	
	2. Base of nem. to rear of theca	26.4	29.5	28.5	
	3. Value of fraction 1 ÷ 2	.19	.37	.31	
" 114	1.	8.2	11.3	9.9	
	2.	27	28.7	28.6	
	3.	.30	.39	.35	
" 177	1.	8.2	11.3	12	9.5
	2.	24	27.8	30.7	32.3
	3.	.34	.40	.39	.29

struthionides may exceed 23 cm. in length. *Aglaophenia* sp. has a particularly stringy, sprawling habit. The character is of no practical value in the present connection.

Aglaophenia sp. — This species differs from *A. struthionides* in no particulars essential to the present discussion.

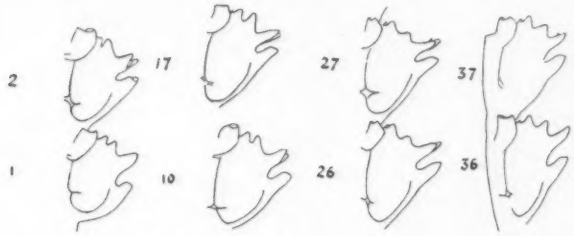

FIG. 6. *A. diegensis*, × 45.

TABLE XII.

Distance from tip of nematophore to rear of theca along perpendicular to axis of internode. Measurements in mm. × 95.

Hydrocladium.	Hydrotheca.							
	1	2	7	8	11	12	16	17
15	24	31	34.5	33	32	31		
56	24.5	30	35.5			40	32	30
74	25	29.5	34.5			37.5		

Hydrocladium 74 is incomplete, hydrotheca 12 not being terminal.

A. diegensis. — Of all the species, this is the most irregular. It agrees in general, however, with *A. octocarpa.* The hydrocladia taper distalward; the first hydrotheca is small and more or less irregular, with short mesial nematophore; the mesial nematophore is most prominent between the extremes of the hydrocladia; and there is a slight lessening of the obliqueness of the hydrothecal openings distalward. Fig. 6 is from a single hydrocladium.

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